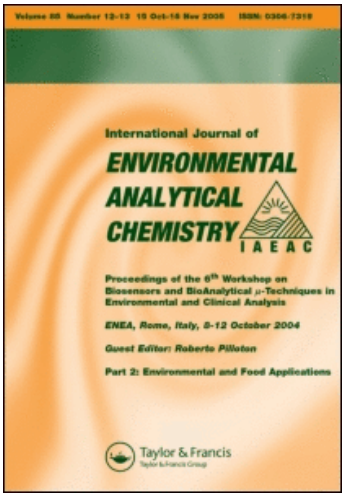

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Anaerobic Degradation of PCB in Soils

Ľ. Haluščarka[a]; Š. Baláž[a]; K. Dercová[a]; E. Benická[b]; J. Krupčík[b]; P. Bielek[c]; G. Lindišolá[a]
[a] Department of Biochemical Technology, Faculty of Chemical Technology, Slovak Technical University, Bratislava, Slovakia [b] Department of Analytical Chemistry, Faculty of Chemical Technology, Slovak Technical University, Bratislava, Slovakia [c] Institute of Soil Fertility Research, Bratislava, Slovakia

To cite this Article Haluščarka, Ľ. , Baláž, Š. , Dercová, K. , Benická, E. , Krupčík, J. , Bielek, P. and Lindišolá, G.(1995) 'Anaerobic Degradation of PCB in Soils', International Journal of Environmental Analytical Chemistry, 58: 1, 327 — 336
To link to this Article: DOI: 10.1080/03067319508033134
URL: http://dx.doi.org/10.1080/03067319508033134

Intern. J. Environ. Anal. Chem., Vol. 58, pp. 327-336
Reprints available directly from the publisher
Photocopying permitted by license only

ANAEROBIC DEGRADATION OF PCB IN SOILS

Ľ. HALUŠKA*, Š. BALÁŽ*, K. DERCOVÁ*, E. BENICKÁ†, J. KRUPČÍK†, P. BIELEK‡ and G. LINDIŠOVÁ*

**Department of Biochemical Technology, Faculty of Chemical Technology, Slovak Technical University, 812 37 Bratislava, Slovakia; †Department of Analytical Chemistry, Faculty of Chemical Technology, Slovak Technical University, 812 37 Bratislava, Slovakia; ‡Institute of Soil Fertility Research, 827 13 Bratislava, Slovakia*

(Received, 10 September 1993; in final form, 4 February 1994)

The anaerobic degradation of PCB in loamy and clayey soils containing indigeneous microflora was studied. The anaerobic conditions were created by an argon atmosphere in the flasks containing soil flooded by a liquid medium with glucose. GC-ECD analysis of soil extracts after 40 day incubation showed, in addition to the concentration changes of the less chlorinated PCB congeners, a significant decrease in the concentration of highly chlorinated congeners in both soils. The results indicate that in both soil types reductive dehalogenation of PCB congeners was encountered.

KEY WORDS: PCB, polychlorinated biphenyls, anaerobic degradation, reductive dehalogenation, soil.

INTRODUCTION

PCB are currently of great concern owing to their recalcitrance to degradation and toxicity. Laboratory studies have now demonstrated that the more highly chlorinated PCB congeners in commercial mixtures can be reductively dechlorinated by anaerobic microorganisms from PCB contaminated sediments.[1-4]. Reductive dehalogenation of highly chlorinated congeners is probably the only mechanism for their biological decay and is the first step for complete mineralization of these compounds by subsequent attack of aerobic microflora.[5-6]

The dehalogenation reactions of PCB have so far been studied mostly in anaerobic sewage sludge and lake sediments. Their occurrence in soils has not been reported. From a practical point of view accidentally polluted soils are of great importance, because they are, due to the high hydrophobicity of PCB, potentially long-time primary source of contamination of waters and water sediments.

Anaerobiosis often dominates biological and chemical processes of flooded and poorly drained soils.[7] Although many anaerobic microsites exist in soil, these are generally occupied by native microbial strains and exogeneously added bacteria are often excluded from these sites.[8] According to Skinner[9], facultative anaerobes represent up to 10% of the total soil

population (i.e. about 10^7 organisms per g soil). Obligate anaerobes mostly exist in dry soils as spores rather than vegetative cells. In poorly drained and flooded soils they would be expected to play a more important role than in the more aerobic dry soils.

The aim of this work was to investigate the influence of anaerobiosis created by the use of an argon atmosphere on degradation of PCB in contaminated soils containing indigeneous microflora.

MATERIALS AND METHODS

Chemicals. A commercial mixture of PCB, Delor 103 (equivalent to Aroclor 1242, Chemko Strážske, Slovakia) containing 40–42% (w/v) of bound chlorine, Delor 106 (equivalent to Aroclor 1260, Chemko Strážske, Slovakia) containing 60% (w/v) of bound chlorine), decachlorobiphenyl (Institute for Drug Research, Modra, Slovakia), n-hexane (Pestiscan, Labscan Ltd, Ireland), acetone (Microchem Bratislava, Slovakia), with purity grade were used.

Soils. Two types of soils were used: type 1 was loamy soil from a locality in Gabčíkovo, Slovakia and type II was a clayey soil from Kvakovce, Slovakia.

Contamination of soils by PCB. In order to ensure homogeneous contamination of soils by these extremely hydrophobic compounds with minimal damage to structure and native microflora of soil the following procedure of contamination was used. Crushed soil (75 g) was flooded by the hexane solution of the mixture of Delor 103 and Delor 106 (2:1, w/w) and decachlorobiphenyl (2% w/w) with the resulting concentration 100 μg PCB per g soil. After evaporation of hexane, soil was thoroughly homogenised and 3 g were mixed with 27 g of intact soil in 50 ml flasks. The resulting concentration was 10 μg PCB per g soil. Deviations of contamination did not exceed 5%.

PCB degradation. PCB contaminated soil (30 g) in 50 ml flasks was flooded by 18 ml of mineral DMA medium[10,11] with glucose (20 g/l). The flasks were closed by a rubber stopper and argon was blowed into them once a week through two needles crossing the stopper. The controls containing sterilized contaminated soil were prepared as follows. To avoid evaporation of PCB, autoclaved (20 min., 120 kPa) intact soil (27 g) was mixed with PCB contaminated soil (3 g, 100 ppm, prepared as described above, except that hexane was evaporated in a sterile laminary box) and sterilized water (18 ml, autoclaving 20 min, 120 kPa). Controls were kept without argon atmosphere.

All experiments were made in several parallel samples and whole flasks were analyzed after 40 day incubation.

PCB extraction. Soil was extracted with acetone (12 ml), thoroughly mixed with the mixture acetone-hexane (2:3 v/v, 30 ml) and dipped for 30 min into an ultrasonic bath. The liquid phase was connected with acetone from the first step and extracted with water (10 ml) to separate acetone and hexane. The whole procedure was repeated 3 times. A portion

of the collected hexane phases (2 ml)was purified on a column (8×30 mm) of activated Florisil, which was then eluted 3 times with 2 ml of hexane. The eluate was concentrated to 2 ml by blowing a stream of argon and analyzed by gas chromatography (GC).

GC analysis. The degradation kinetics of the individual PCB congeners were monitored by GC (HP 5890) with H_2 as carrier gas (60 kPa, 1,5 ml/min, split-splitless inlet mode), equipped with an electron capture detector (ECD) (280°C, make up gas N_2 at 60 ml/min), using a 50 m × 0.32 mm I.D. fused silica capillary column with a nonpolar stationary phase HP 1 (thickness 0,17 μm). Temperature conditions: injector 250°C, column 45°C (0.5 min)—20°C/min—150°C—2°C/min—250°C (6 min). Identification of peaks and their calibration was made according to Krupčik *et al.*[12] The reproducibility of the quantitative analysis was controlled using the standard solution of Delor 103 and Delor 106 (7.5 μg/ml) in hexane. Relative deviations for congeners which did not interfere with the background were around 3%.

RESULTS AND DISCUSSION

Various types of soils differ in physical (structure and texture, sorption quality, pH, redox potential), chemical (content of total organic carbon, structure and content of humic acids, content of nutrients or toxic compounds), and biological characteristics (the spectrum and concentration of microorganisms) which influence significantly the degradation processes in soil. For this reason two different soils were chosen for all experiments: a loamy soil (type I), rich in organic carbon, humic acids and microorganisms and, in all the aspects, a poor clayey soil (type II).

The degradation of PCB congeners was monitored by GC-ECD analysis. Figure 1 shows the amount of PCB congeners remaining in both types of soil, after 40 day incubation in comparison with the controls analyzed at the same time. The peaks were, for the sake of simple orientation, marked with numbers 1–87. Details about PCB congeners eluted in individual peaks are given in Table 1. As can be seen in Figure 2, the PCB degradation profile in soil II is in agreement with expectation for the incubation under anaerobic conditions: the content of highly chlorinated congeners decreased, in contrast to the less chlorinated congeners, which can be formed from the higher chlorinated congeners and their concentration increased. For example, as shown in Table 2, while after 40 day incubation the chromatographic peak containing 2,2′-dichlorobiphenyl and 2,6-dichlorobiphenyl is at the level of 118% as compared to the control, the concentration of decachlorobiphenyl decreased to only 68%. Decachlorobiphenyl has no chlorine-free sites for aerobic attack, and so probably the only explanation for the elimination of this congener is its reductive dechlorination. The summation of the data shows that 10.6% of total chlorine and 7.5% of ortho chlorine was removed during 40 day incubation. This suggests that meta and para chlorines, associated with toxicity of PCB, are removed preferentially.

Degradation profile in soil I is rather different. It is interesting, that besides the expected decrease of higher chlorinated PCB, di-, tri- and tetra-CB also disappear (Figure 3). Although anaerobic processes attacking the biphenyl ring have been reported[6], aerobic degradation in

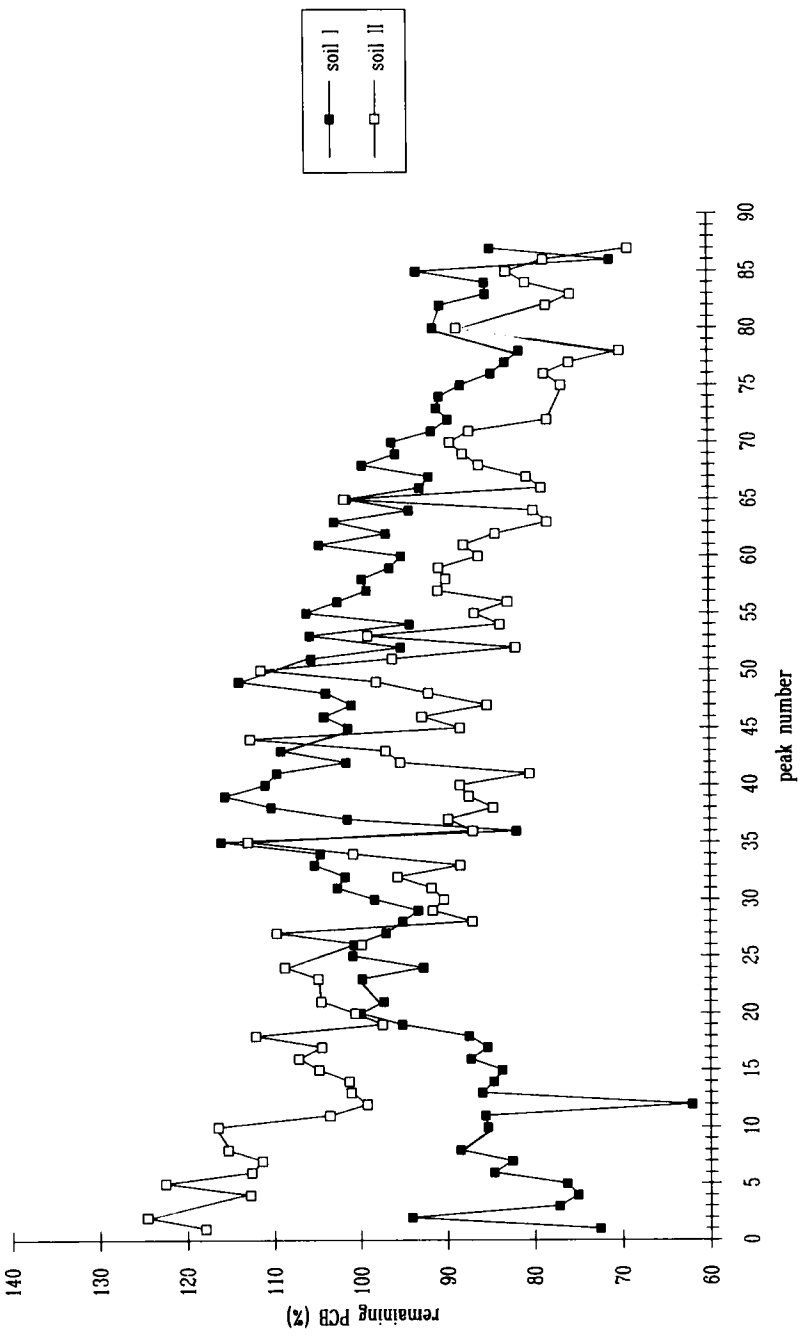

Figure 1 Profile of remaining PCB congeners in soil I (—■—) and soil II (—□—) after 40 day incubation with argon atmosphere in comparison to the controls at the same time. Peak numbers correspond with those indicated in Table 1.

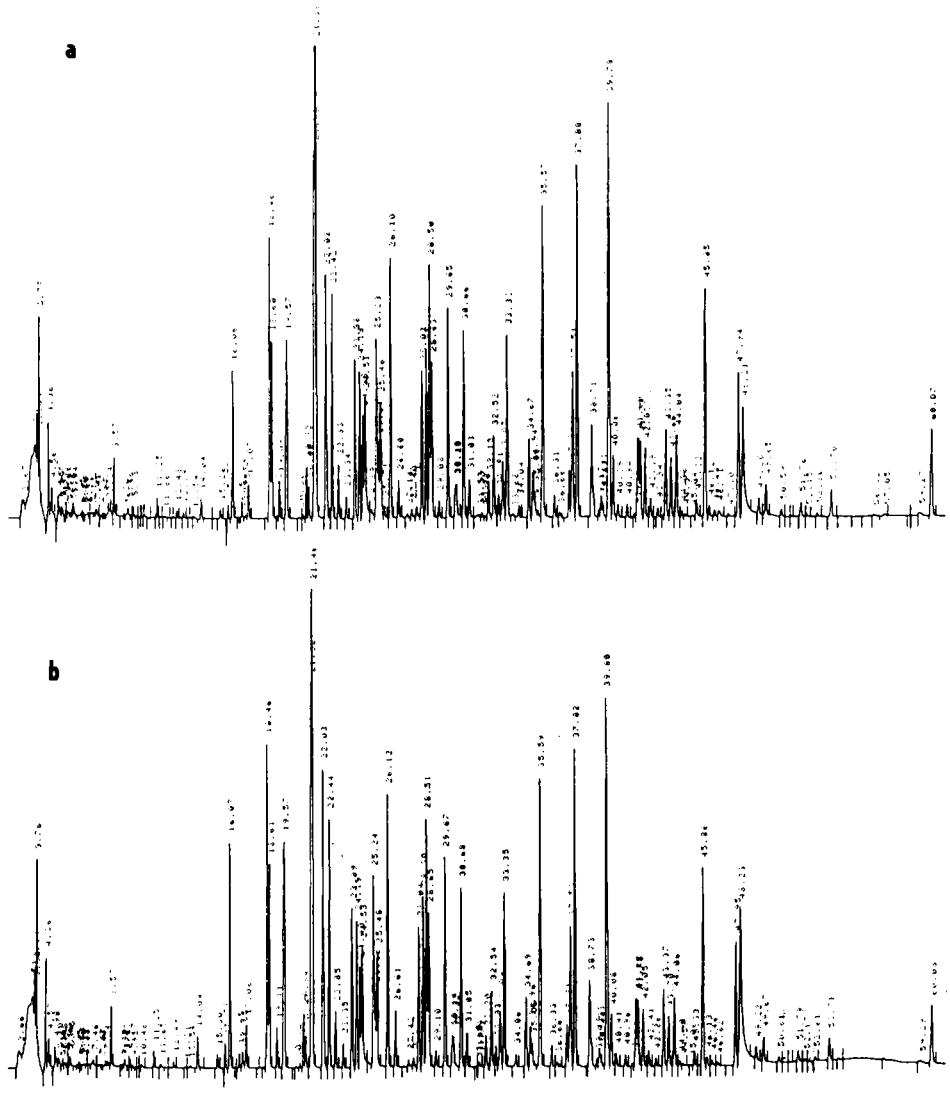

Figure 2 Chromatogram of PCB congeners remaining in soil II after 40 days of incubation with argon atmosphere (b) as compared with the control at the same time (a).

the first days of the experiment cannot be excluded. Soil I is porous and contains initially more oxygen than the clayey soil II. Therefore, the creation of anaerobic conditions by simple blowing argon above the soil surface might be more time-consuming.

The experiments described above might suggest, that only minimal changes of indigenous anaerobic microflora are needed for PCB degradation, or suitable microflora is already

Table 1 Peak number, retention time, IUPAC number and chlorine atom position of the individual congeners of PCB (Delor 103 and Delor 106).

Peak number	*Retention time*	*IUPAC*	*Chlorine atom position*
1	14.03	4, 10	2, 2′; 2, 6
2	15.29	7, 9	2, 4; 2, 5
3	15.77	6	2, 3′
4	16.06	5, 8	2, 3; 2, 4′
5	17.07	19	2, 2′, 6
6	18.46	18	2, 2′, 5
7	18.60	15, 17	4, 4′; 2, 2′, 4
8	19.10	24, 27	2, 3, 6; 2, 3′, 6
10	19.57	16, 32	2, 2′, 3; 2, 4′, 6
11	20.83	26	2, 3′, 5
12	20.97	25	2, 3′, 4
13	21.31	31	2, 4′, 5
14	21.39	28	2, 4, 4′
15	22.02	33, 53	2′, 3, 4; 2, 2′, 5, 6′
16	22.42	22, 51	2, 3, 4′; 2, 2′, 4, 6′
17	22.84	45	2, 2′, 3, 6
18	23.34	46	2, 2′, 3, 6′
19	23.88	52	2, 2′, 5, 5′
20	24.18	49	2, 2′, 4, 5′
21	24.38	48	2, 2′, 4, 5
22	24.50	47	2, 2′, 4, 4′
23	25.22	44	2, 2′, 3, 5′
24	25.34	37	3, 4, 4′
25	25.46	42	2, 2′, 3, 4′
26	26.10	41, 64	2, 2′, 3, 4; 2, 3, 4′, 6
		71, 72	2, 3′, 4′, 6; 2, 3′, 5, 5′
27	26.60	40	2, 2′, 3, 3′,
28	27.40	67	2, 3′, 4, 5
29	27.70	63	2, 3, 4′, 5
30	28.02	74, 94	2, 4, 4′, 5; 2, 2′, 3, 5, 6′
31	28.28	70, 76	2, 3′, 4′, 5; 2′, 3, 4, 5
32	28.50	66	2, 3′, 4, 4′,
33	29.08	55, 91	2, 3, 3′, 4; 2, 2′, 3, 4′, 6
34	29.65	56, 60	2, 3, 3′, 4′; 2, 3, 4, 4′
35	30.12	84, 92	2, 2′, 3, 3′, 6; 2, 2′, 3, 5, 5′
36	30.20	89	2, 2′, 3, 4, 6′
37	30.66	90, 101	2, 2′, 3, 4′, 5; 2, 2′, 4, 5, 5′
38	31.03	99, 113	2, 2′, 4, 4′, 5; 2, 3, 3′, 5, 6
39	32.18	97	2, 2′, 3′, 4, 5
40	32.52	87	2, 2′, 3, 4, 5
41	32.81	111	2, 3, 3′, 5, 5′
42	33.06	148	2, 2′, 3, 4′, 5, 6′
43	33.33	110	2, 3, 3′, 4′, 6
44	34.04	82	2, 2′, 3, 3′, 4
45	34.66	151	2, 2′, 3, 5, 5′, 6
46	34.94	144	2, 2′, 3, 4, 5′, 6
47	35.06	135	2, 2′, 3, 3′, 5, 6′

Table 1 *continued*

Peak number	*Retention time*	*IUPAC*	*Chlorine atom position*
48	35.57	118	2, 3′, 4, 4′, 5
		149	2, 2′, 3, 4′, 5′, 6
49	36.31	134	2, 2′, 3, 3′, 5, 6
50	36.78	131	2, 2′, 3, 3′, 4, 6
51	28.61	95	2, 2′, 3, 5′, 6
52	37.29	146	2, 2′, 3, 4′, 5, 5′
53	37.51	132	2, 2′, 3, 3′, 4, 6′
54	37.79	153	2, 2′, 4, 4′, 5, 5′
55	38.71	141	2, 2′, 3, 4, 5, 5′
		179	2, 2′, 3, 3′, 5, 6, 6′
56	39.17	137	2, 2′, 3, 4, 4′, 5
57	39.31	130	2, 2′, 3, 3′, 4, 5′
58	39.41	176	2, 2′, 3, 3′, 4, 6, 6′
59	39.77	138	2, 2′, 3, 4, 4′, 5′
		163	2, 3, 3′, 4′, 5, 6
		164	2, 3, 3′, 4′, 5′, 6
60	40.06	158	2, 3, 3′, 4, 4′, 6
61	40.38	129	2, 2′, 3, 3′, 4, 5
62	40.93	178	2, 2′, 3, 3′, 5, 5′, 6
63	41.38	175	2, 2′, 3, 3′, 4, 5′, 6
64	41.59	182, 187	2, 2′, 3, 3′, 4; 2, 2′, 3, 4, 5′
		159	2, 3, 3′, 4, 5, 5′
65	41.72	128	2, 2′, 3, 3′, 4, 4′
66	42.02	183	2, 2′, 3, 4, 4′, 5′, 6
67	42.38	167	2, 3′, 4, 4′, 5, 5′
68	42.84	185	2, 2′, 3, 4, 5, 5′, 6
69	43.34	174	2, 2′, 3, 3′, 4, 5, 6′
70	43.67	177	2, 2′, 3, 3′, 4′, 5, 6
71	44.03	156	2, 3, 3′, 4, 4′, 5
		171	2, 2′, 3, 3′, 4, 4′, 6
72	43.37	202	2, 2′, 3, 3′, 5, 5′, 6, 6′
73	44.56	173	2, 2′, 3, 3′, 4, 5, 6
74	45.02	200	2, 2′, 3, 3′, 4, 5′, 6, 6′
75	45.31	172	2, 2′, 3, 3′, 4, 5, 5′
		192	2, 2′, 3, 3′, 4, 5, 5′
76	45.84	180	2, 2′, 3, 4, 4′, 5, 5′
77	46.11	193	2, 3, 3′, 4′, 5, 5′, 6
78	46.43	191	2, 3, 3′, 4, 4′, 5, 6
79	46.80	199	2, 2′, 3, 3′, 4′, 5, 6, 6′
80	47.92	170	2, 2′, 3, 3′, 4, 4′, 5
81	48.18	190	2, 3, 3′, 4, 4′, 5, 6
82	49.19	201	2, 2′, 3, 3′, 4′, 5, 5′, 6
83	49.64	196	2, 2′, 3, 3′, 4, 4′, 5, 6′
		203	2, 2′, 3, 4, 4′, 5, 5′, 6
84	50.58	189	2, 3, 3′, 4, 4′, 5, 5′
85	51.77	195	2, 2′, 3, 3′, 4, 4′, 5, 6
86	53.68	194	2, 2′, 3, 3′, 4, 4′, 5, 5′
87	60.04	209	2, 2′, 3, 3′, 4, 4′, 5, 5′, 6, 6′

Table 2 The amount of PCB congeners (%) remaining in both types of soils after 40 day incubation as compared to the controls at the same time. Twenty significant chromatographic peaks have been chosen, details being given in Table 1.

Peak No.	*IUPAC*	*Avg. No. of Cl*		*Soil I (%)*	*Soil II (%)*
		Total	*Ortho*		
1	4, 10	2	2	73	118
4	5, 8	2	1	75	113
6	18	3	2	85	113
10	16, 32	3	2	86	117
19	52	4	2	95	98
26	41, 64, 71, 72	4	1, 75	101	100
34	56, 60	4	1	105	100
37	90, 101	5	2	101	89
43	110	5	2	109	96
48	118, 149	5, 5	2, 75	103	91
45	151	6	3	101	88
54	153	6	2	94	83
59	138, 163, 164	6	2	96	90
66	183	7	3	92	78
69	174	7		95	87
76	180	7	2	84	78
80	170	7	2	91	88
83	196, 203	8	3	85	75
86	194	8	2	71	78
87	209	10	4	84	68

encountered in soil. This is somewhat surprising, especially if it is considered that the soils were not polluted previously. However, there is also another explanation for the dehalogenation observed in the experiments in which the microflora was not in contact with PCB for a long time. Reductive dehalogenation may represent both enzymatic and nonenzymatic activities. For example, Assaf-Anid *et al.*[13] observed that 2, 3, 4, 5, 6-pentachlorobiphenyl was reductively dechlorinated in an aqueous biomimetic model system containing vitamin B_{12}. An increase in the substrate level (glucose in our case) would lead to an increase in the total amount of coenzymes (as a consequence of the increase in the total amount of microorganisms, which synthetize the coenzyme derivative of vitamin B_{12} for the use in normal metabolic functions) and also in the number of electrons available for reductive dechlorination. This is in agreement with observations made by Nies and Vogel[14], who investigated the effects of organic substrates on dechlorination of Aroclor 1242 in anaerobic sediments. Speculation, that nonenzymatic dechlorination may play a significant role in elimination of highly chlorinated PCB is supported by the frequent occurence of PCB mixtures with domination of lightly chlorinated congeners under appropriate conditions in aquatic sediments, and also by the fact, that aryl reductive dehalogenation has been found in a variety of undefined anaerobic conditions, but only in single pure culture *Desulfomonile tiedjei*.[15,16]

Anaerobiosis is a frequent phenomenon in poorly drained and flooded soils and examination of environmental effects which could influence biotic and abiotic reductive dehalogenation and their optimization will certainly help in decontamination of PCB polluted soils.

Figure 3 Remaining amounts of PCB congeners in soil I (—□—) and soil II (—■—) after 40 day incubation with argon atmosphere as dependent on the chlorine number.

Acknowledgements

This work was made possible by Grant No. 1/990969/93 from the Slovak Grant Agency.

References

1. P. J. Morris, W. W. Mohn, J. F. Quensen III, J. M. Tiedje and S. A. Boyd, *Appl. Environ. Microbiol.*, **58,** 3088–3094 (1992).
2. J. F. Quensen III, S. A. Boyd and J. M. Tiedje, *Appl. Environ. Microbiol.*, **56,** 2360–2369 (1990).
3. H. M. Van Dort, and D. L. Bedard, *Appl. Environ. Microbiol.*, **57,** 1576–1578 (1991).
4. D. Ye, J. F. Quensen III, J. M. Tiedje and S. A. Boyd, *Appl. Environ. Microbiol.*, **58,** 1110–1114 (1992).
5. S. W. Hooper, Ch. A. Pettigrew and G. R. Sayler, *Environ. Toxicol. Chem.*, **9,** 655–667 (1990).
6. D. A. Abramowicz, *Crit. Rev. Biotechnol.*, **10,** 241–251 (1990).
7. J. M. Tiedje, A. J. Sexstone, T. B. Parkin, N. P. Revsbech and D. R. Shelton, *Plant and Soil*, **76,** 197–212 (1984).
8. R. H. Kaake, D. J. Roberts, T. O. Stevens, R. L. Crawford and D. L. Crawford, *Appl. Environ. Microbiol.*, **58,** 1683–1689 (1992).
9. F. A. Skinner, in: *Soil microbiology a critical review* (N. Walker, ed. Butterworth and Co., London, 1975) pp. 1–19.
10. S. J. Pirt, *J. Gen. Microbiol.*, **47,** 181–197 (1967).
11. K. Dercová, Š. Baláž, Ľ. Haluška, V. Horňák and V. Holecová, *Intern. J. Environ. Anal. Chem.* (1994) in press.
12. J. Krupčík, A. Kočan, J. Petrík, P. A. Leclercq and K. Ballschmiter, *Chromatographia* **33,** 514–520 (1992).
13. N. Assaf-Anid, L. Nies and T. M. Vogel, *Appl. Environ. Microbiol*, **58,** 1057–1060 (1992).
14. L. Nies and T. M. Vogel, *Appl. Environ. Microbiol.* **56,** 2612–2617 (1990).
15. W. W. Mohn and K. J. Kennedy, *Appl. Environ. Microbiol.* **58,** 1367–1370 (1992).
16. W. W. Mohn and J. M. Tiedje, *Microbiol. Rev.*, **56,** 482–507 (1992).